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Supplemental Material

Air Pollution–Mediated Susceptibility to Inflammation and Insulin Resistance: Influence of CCR2 Pathways in Mice

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Table of Contents

Supplemental Material, Table S1. Elemental constituents of air from OASIS in Columbus, Ohio, December 2011 to March 2012 by energy-dispersive X-ray fluorescence.

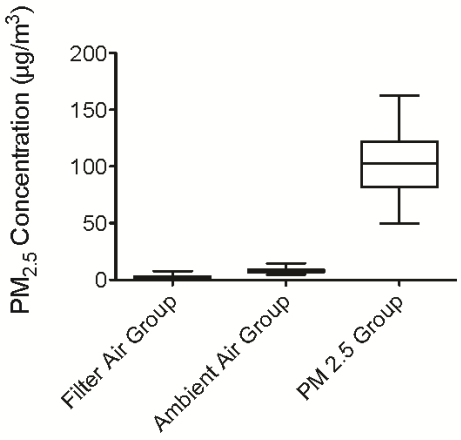
Elements	Ambient Air	Filtered Air	$PM_{2.5}$ Air
S	672.0 ± 146.6	-0.1 ± 43.6	6020.0 ± 3292.9
Ca	74.0 ± 22.1	66.2 ± 40.5	545.7 ± 304.5
Na	72.6 ± 34.2	47.0 ± 29.8	412.4 ± 247.5
Fe	42.0 ± 21.0	21.1 ± 23.2	353.8 ± 219.1
K	39.3 ± 15.0	22.8 ± 18.0	263.8 ± 159.1
Zn	21.1 ± 15.5	7.8 ± 12.5	182.7 ± 180.1
Mg	19.9 ± 7.8	11.2 ± 5.2	144.9 ± 79.6
Al	16.1 ± 10.1	18.4 ± 22.6	142.5 ± 126.9
P	12.8 ± 8.9	14.0 ± 5.4	86.3 ± 93.6
Pb	3.2 ± 1.4	0.3 ± 0.9	23.8 ± 15.5
Cu	2.2 ± 1.1	1.5 ± 2.2	17.8 ± 10.4
Ba	2.2 ± 0.7	0.6 ± 0.6	17.6 ± 9.8
Mn	1.8 ± 1.0	0.5 ± 0.3	16.0 ± 11.2
Cr	2.2 ± 0.5	5.0 ± 1.9	6.4 ± 3.0
Se	0.6 ± 0.2	0.0 ± 0.0	5.8 ± 3.4
Ti	0.6 ± 0.2	0.1 ± 0.1	5.2 ± 2.9
Sb	0.6 ± 0.1	0.0 ± 0.0	4.4 ± 2.4
Sr	0.4 ± 0.2	0.2 ± 0.1	3.6 ± 2.2
As	*0.4 ± 0.1*	*0.0 ± 0.0*	*3.4 ± 1.9*
Mo	0.4 ± 0.2	0.2 ± 0.3	2.8 ± 1.5
Ni	0.2 ± 0.3	0.1 ± 0.5	1.8 ± 1.5
V	0.2 ± 0.1	0.0 ± 0.0	1.4 ± 0.9
Cd	0.1 ± 0.1	0.1 ± 0.1	0.9 ± 0.5
Rb	0.1 ± 0.0	0.0 ± 0.0	0.6 ± 0.4
Ce	0.0 ± 0.0	0.0 ± 0.0	0.4 ± 0.3
La	0.0 ± 0.0	0.0 ± 0.0	0.3 ± 0.2
Co	0.0 ± 0.0	0.1 ± 0.0	0.2 ± 0.1

Units are ng/mg; n = 14 filters. Data are means ± SD.

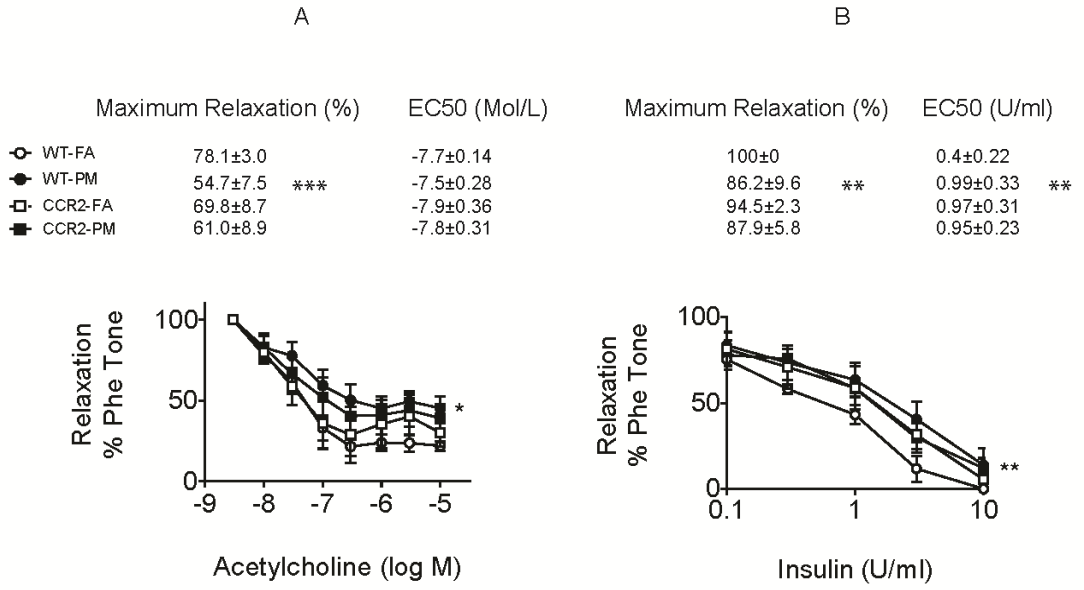
Supplemental Material, Table S2. Effect of $PM_{2.5}$ exposure on circulating inflammatory cytokines in WT and CCR2-/- mice fed an HFD.

	Groups			
Items	**WT-FA**	**WT-PM**	**CCR2-FA**	**CCR2-PM**
TNFα (pg/ml)	9.7 ± 0.6	15.6 ± 3.0	12.4 ± 2.9	11.4 ± 1.4
IL-6 (pg/ml)	5.1 ± 0.4	11.2 ± 5.1	5.3 ± 0.5	9.0 ± 1.8
MCP-1 (pg/ml)	32.0 ± 2.2	33.0 ± 2.2	111.2 ± 21.1***	120.4 ± 14.7***###
IFNγ (pg/ml)	1.8 ± 0.2	2.4 ± 0.8	2.1 ± 0.4	1.8 ± 0.2
IL-12 p70 (pg/ml)	10.8 ± 1.3	8.5 ± 2.2	15.1 ± 4.0	8.4 ± 0.9

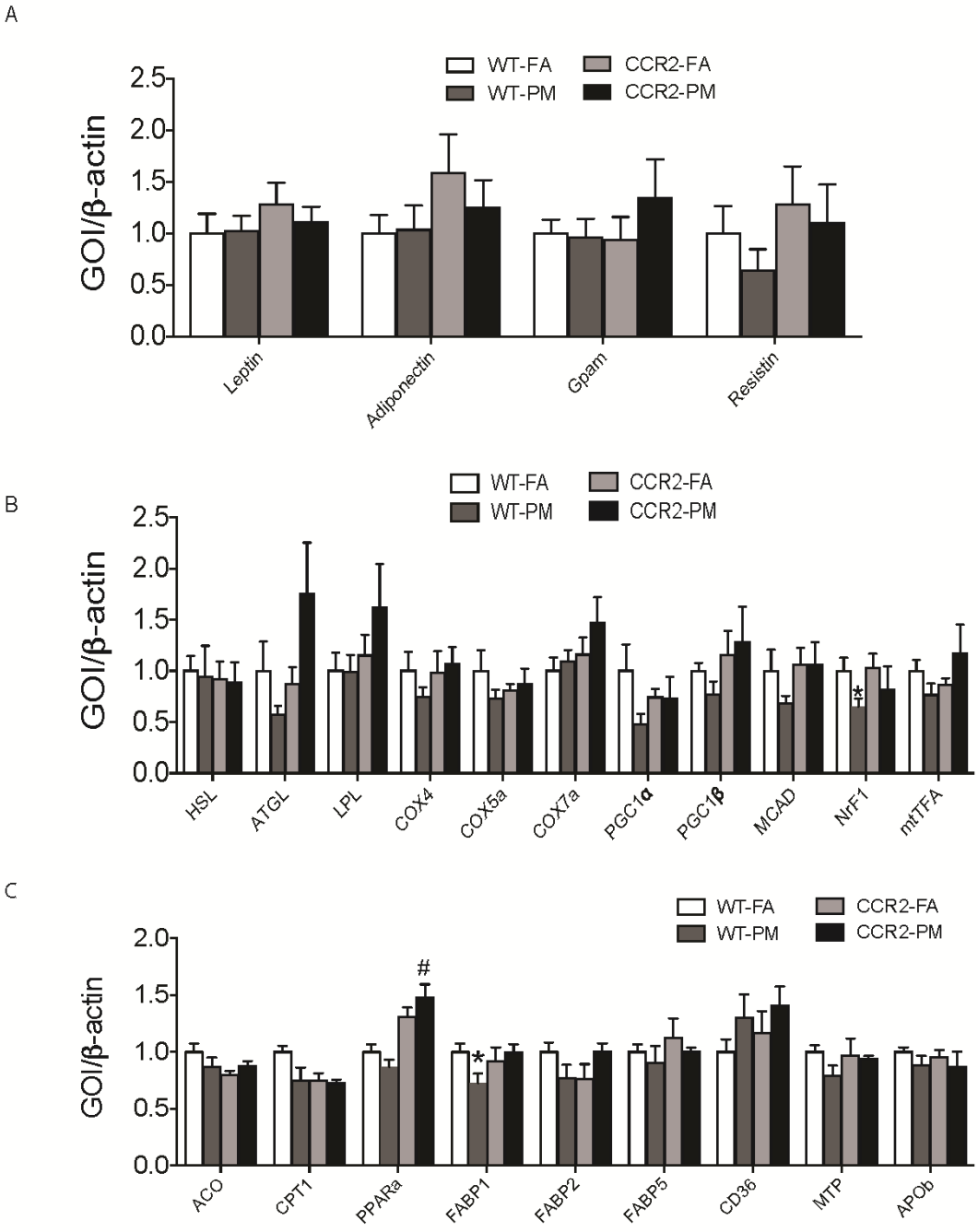
Note: ***$p < 0.001$ compared with WT-FA group, ###$p < 0.001$ compared with WT-PM group. Data are expressed as means ± SEM. n = 7-9 per group.



Supplemental Material, Figure S1. $PM_{2.5}$ concentration to which mice were exposed at the study site. Data are means ± SD of 9-12 filters.

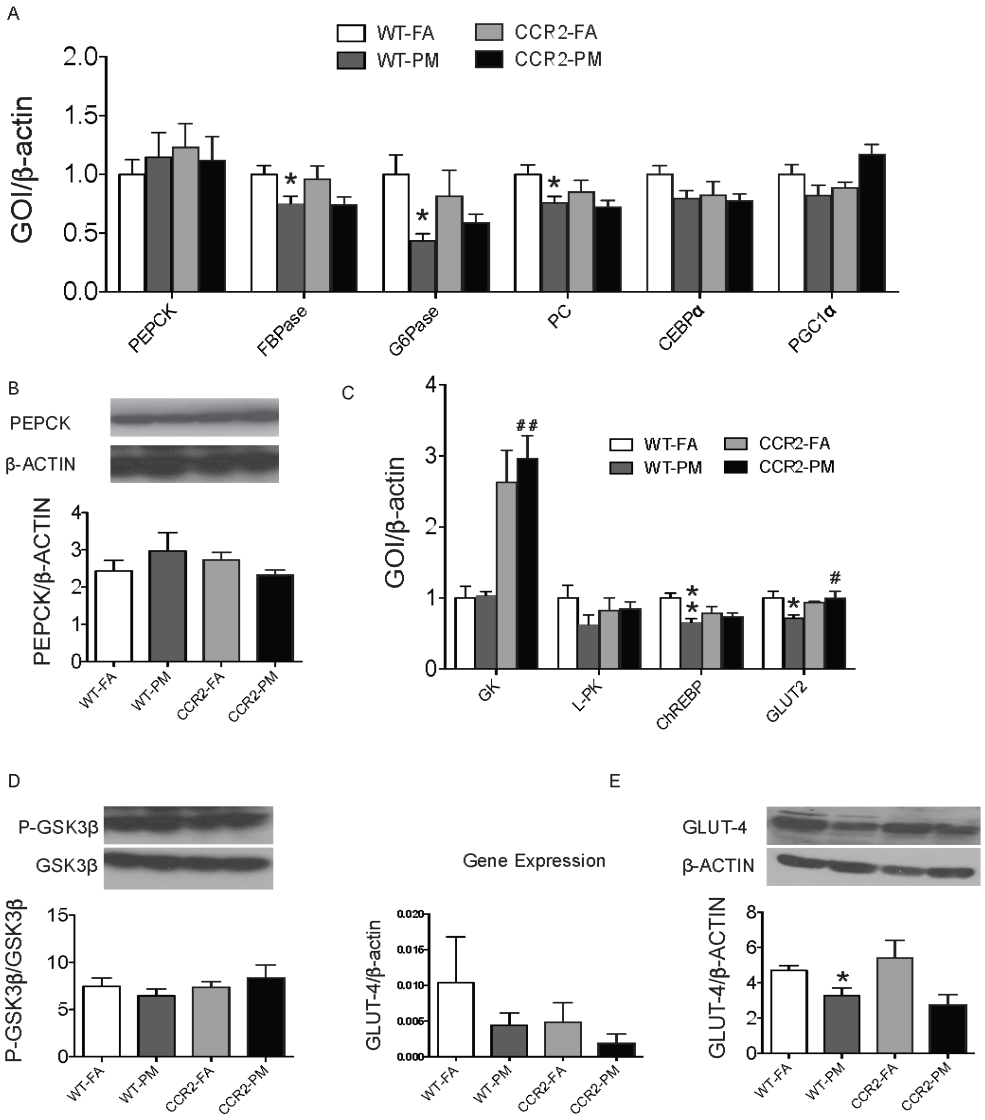


Supplemental Material, Figure S2. Effect of $PM_{2.5}$ exposure on endothelium-dependent vascular relaxation in aorta from HFD-fed mice. A-B, Maximum relaxation, EC_{50} and dose-response to acetylcholine (A) and insulin (B) in aortic rings precontracted with phenylephrine. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ when WT-PM compared to WT-FA group. n = 7-9 per group.



Supplemental Material, Figure S3. Effect of $PM_{2.5}$ exposure on mRNA levels of genes in VAT and liver of HFD-fed mice. A, mRNA levels of genes involved in adipocyte function in VAT. B, mRNA levels of genes involved in lipolysis and mitochondrial oxidation and/or biogenesis in VAT. Hormone sensitive lipase (HSL), Adipose triglyceride lipase (ATGL), Lipoprotein lipase

(LPL) are involved in lipolysis. Cytochrome c oxidase subunit VI (COX4), Va (COX5a), VIIa (COX7a), peroxisome proliferator-activated receptor gamma coactivator 1 α and β (PGC1α, PGC1β), and medium-chain acyl-CoA dehydrogenase (MCAD) are involved in mitochondrial oxidation. Nuclear respiratory factor 1 (NRF1) and mitochondrial transcription factor A (mtTFA) are involved in mitochondrial biogenesis. C, mRNA levels of genes involved in lipid metabolism in the liver. Acyl-CoA oxidase (ACO), Carnitine palmitoyltransferase 1 (CPT-1), and PPARα are involved in β-oxidation. Fatty acid binding protein 1 (FABP1), FABP2, FABP5, and CD36 are involved in fatty acid uptake. Microsomal triglyceride transfer protein (MTP) and Apolipoprotein b (Apob) are involved in VLDL secretion.*$p < 0.05$ when WT-PM compared to WT-FA group, #$p < 0.05$ when CCR2-PM compared to WT-PM group. n = 7-9 per group.



Supplemental Material, Figure S4. Effect of $PM_{2.5}$ exposure on glucose metabolism-related signals in the liver and muscle of HFD-fed mice. A, mRNA levels of gluconeogenesis-related genes in the liver. B and D, Western blotting for PEPCK (B) and phospho-GSK3β/total GSK3β (D) in the liver. C, mRNA levels of glycolysis-related gene in the liver. E, mRNA and protein levels of GLUT-4 expression in the skeletal muscle. *$p < 0.05$ when WT-PM compared to WT-FA group, #$p < 0.05$, ## $p < 0.01$ when CCR2-PM compared to WT-PM group. n = 5-9 per group.